Vascular Biology in Genetically Altered Mice
Smaller Vessels, Bigger Insight

Frank M. Faraci, Curt D. Sigmund

Manipulation of genes in the mouse genome to produce transgenic or gene-targeted animals represents a powerful experimental tool with which to study the role of specific gene products in complex physiological systems. Because of the power of studying genetically altered mice, a major effort has begun to incorporate the use of these models in studies of the cardiovascular system, including vascular biology.

The present review will summarize progress that has been made in studies of vascular biology in mice. A major focus of the discussion will be studies of vascular function. At present, the majority of studies of vascular function in genetically altered mice have been related to the role of endothelium. For this reason, we will highlight studies related to endothelium and nitric oxide (NO, a major vasoactive substance produced by endothelium) as examples of the types of studies that are being done in mice and of the new insight into the regulation of blood vessels that is emerging.

The article will also summarize advantages and limitations of using genetically altered mice as well as new insights obtained from such studies in the areas of signal transduction and pathophysiology. In addition, we will summarize potential directions and methodologies for future studies of vascular biology in mice.

Use of Normal Mice for Studies of Vascular Biology

The methodology to produce targeted gene disruption in mammalian cells that subsequently contribute to the germ line was developed in the mid to late 1980s. Before the age of producing genetically altered mice, studies of vascular function in mice appeared only occasionally in the literature. Examples are studies of vascular responses in mice made hypertensive by psychosocial stress. Even in more recent years, mice have only occasionally been used for studies of normal vascular function and for examining the effects of hypercholesterolemia (produced by a high fat diet) or diabetes (produced by use of streptozotocin or alloxan) on blood vessels. Exceptions to this trend were the studies by Rosenblum, who used mice exclusively over the past 3 decades and published many articles dealing with cerebral vascular function.

Advantages of Genetically Altered Mice

Mice that overexpress or are lacking in expression of selected genes are excellent models for establishing the functional importance of a particular gene product. Homologous recombination using embryonic stem cells (“gene targeting”) is a powerful investigative tool being widely used in many disciplines. At present, the mouse is the only mammalian species in which gene targeting can first be performed and then be successfully transmitted to the germ line. In addition to studies of loss of gene function, these animals often represent models of human inherited or acquired diseases. The phenotypes resulting from such alterations have ranged from the predictable to the surprising. In some cases, mice carrying a disrupted gene appear phenotypically normal. Findings such as the latter may result from redundancy in the genome and/or the expression of compensatory mechanisms.

One of the great strengths of the gene-targeting technique is that it can eliminate many problems present in other, more commonly used models. This includes the limited specificity of pharmacological agents (eg, enzyme inhibitors or receptor antagonists). A good example of such a limitation in relation to vascular biology is studies of the NO system using inhibitors of NO synthase (the enzyme that converts L-arginine and molecular oxygen to NO and L-citrulline). It is now known that there are 3 major isoforms of NO synthase. These enzymes are products of separate genes and are designated neuronal, endothelial, and inducible isoforms of NO synthase (nNOS, eNOS, and iNOS, respectively). Although pharmacological inhibitors of NO synthase such as N^6-monomethyl-L-arginine have been very useful in examining the role of NO in vascular biology, a major limitation exists because this analogue of L-arginine (and most inhibitors of NO synthase) nonselectively inhibits all isoforms of the enzyme (eNOS, iNOS, and nNOS). For example, there are no selective inhibitors of eNOS. Thus, it is difficult to study effects of selective inhibition of single isoforms of NO synthase. In addition, when inhibitors of NO synthase are used, there are often uncertainties regarding tissue or cellular access as well as the extent of enzyme inhibition. Finally, some studies have suggested that inhibitors of NO synthase may have effects unrelated to inhibition of that enzyme.
A major strength of the gene-targeting approach is that it allows the use of a precise genetic alteration to study complex responses in blood vessels or in intact animals. Gene targeting offers a level of specificity that traditional pharmacology can rarely (if ever) achieve.

In addition to studies of gene deletion in mice generated through gene targeting, the generation of transgenic mice that overexpress a selected gene is also a common approach. With this approach, one can study the effects of overexpression of a candidate gene that may contribute to normal vascular regulation or vascular dysfunction under pathophysiological conditions. A transgenic animal is one that contains a segment of exogenous genetic material that is stably incorporated into the recipient genome. In contrast to deletion of a gene through gene targeting, overexpression of genes through the use of transgenics can be performed in other species. An example of studies involving the cardiovascular system is the transgenic rat that overexpresses the mouse Ren2 gene (TGR(mRen2)27) and is chronically hypertensive.

### Endothelial Function

A major focus of research in vascular biology relates to the role of endothelium in health and disease. Thus, it is perhaps not surprising that the majority of studies of vascular function in genetically altered mice are related to endothelial function (Tables 1 and 2).

Studies to date suggest that endothelial function is generally similar in blood vessels from normal mice compared with blood vessels from other species. For example, acetylcholine (the classic endothelium-dependent agonist) produces relaxation of the mouse aorta, carotid, pulmonary, coronary, and mesenteric arteries; cerebral arterioles; cerebral circulation; renal and mesenteric arteries; cerebral circulation; hindlimb circulation. Although transgenic research can be performed in other species, the vast majority of this work is also performed with the use of mice because of the greater relative technical ease of manipulating the mouse embryo.

### Table 1. Mouse Models in Which Vascular Function Has Been Studied After Targeted Gene Deletion

<table>
<thead>
<tr>
<th>Deleted Gene</th>
<th>Blood Vessel or Circulation</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td>Aorta; carotid, pulmonary, coronary, and mesenteric arteries; cerebral arterioles; cerebral circulation</td>
<td>26, 28, 31, 34, 37, 41, 44, 45, 54–56, 125</td>
</tr>
<tr>
<td>iNOS</td>
<td>Carotid artery; cremaster circulation</td>
<td>109, 111</td>
</tr>
<tr>
<td>nNOS</td>
<td>Cerebral arterioles; cerebral circulation; hindlimb circulation</td>
<td>41, 161–163</td>
</tr>
<tr>
<td>cGKI</td>
<td>Aorta</td>
<td>70</td>
</tr>
<tr>
<td>VASP</td>
<td>Aorta</td>
<td>82</td>
</tr>
<tr>
<td>Sarco(endo)plasmic reticulum</td>
<td>Aorta</td>
<td>29</td>
</tr>
<tr>
<td>Ca(^{2+})-ATPase</td>
<td>Aorta; renal and mesenteric arteries</td>
<td>36, 64</td>
</tr>
<tr>
<td>Phospholamban</td>
<td>Aorta</td>
<td>67, 164</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>Aorta</td>
<td>42, 165</td>
</tr>
<tr>
<td>Guanylate cyclase-A natriuretic peptide receptor</td>
<td>Aorta; pulmonary circulation</td>
<td>166, 167</td>
</tr>
<tr>
<td>α1-adrenergic receptor</td>
<td>Aorta</td>
<td>168</td>
</tr>
<tr>
<td>Endothelin-A receptor</td>
<td>Renal and mesenteric circulation</td>
<td>169</td>
</tr>
<tr>
<td>Endothelin-B receptor</td>
<td>Aorta; renal and mesenteric circulation</td>
<td>43, 169</td>
</tr>
<tr>
<td>Bradykinin-B(_2) receptor</td>
<td>Aorta; mesenteric circulation</td>
<td>63, 170</td>
</tr>
<tr>
<td>Prostacyclin receptor</td>
<td>Aorta</td>
<td>171</td>
</tr>
<tr>
<td>Angiotensin II(_A) receptor</td>
<td>Renal circulation</td>
<td>172</td>
</tr>
<tr>
<td>Angiotensin II(_B) receptor</td>
<td>Aorta</td>
<td>173</td>
</tr>
<tr>
<td>Insulin receptor substrate-1</td>
<td>Aorta</td>
<td>174</td>
</tr>
<tr>
<td>IL-10</td>
<td>Carotid artery; cremaster circulation</td>
<td>108, 112, 113</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Mesenteric artery</td>
<td>35</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Mesenteric artery</td>
<td>35</td>
</tr>
<tr>
<td>CD11/CD18</td>
<td>Mesenteric artery</td>
<td>35</td>
</tr>
<tr>
<td>Fibroblast growth factor-2</td>
<td>Aorta</td>
<td>175</td>
</tr>
<tr>
<td>apoE</td>
<td>Aorta; coronary arteries</td>
<td>27, 30, 32</td>
</tr>
<tr>
<td>apoE and LDL receptor</td>
<td>Aorta; coronary arteries</td>
<td>27, 32</td>
</tr>
<tr>
<td>Elastin</td>
<td>Aorta</td>
<td>176</td>
</tr>
<tr>
<td>Inwardly rectifying K(^+) channel</td>
<td>Cerebral artery</td>
<td>131</td>
</tr>
</tbody>
</table>
TABLE 2. Transgenic Mouse Models in Which Vascular Function Has Been Studied

<table>
<thead>
<tr>
<th>Overexpressed Gene</th>
<th>Blood Vessel or Circulation</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td>Aorta</td>
<td>57</td>
</tr>
<tr>
<td>Atrial natriuretic factor</td>
<td>Coronary arteries</td>
<td>33</td>
</tr>
<tr>
<td>Renin and angiotensinogen</td>
<td>Aorta; carotid arteries</td>
<td>90, 91</td>
</tr>
<tr>
<td>Amyloid precursor protein</td>
<td>Cerebral circulation</td>
<td>114, 115</td>
</tr>
<tr>
<td>Amyloid precursor protein and CuZn-SOD</td>
<td>Cerebral circulation</td>
<td>115</td>
</tr>
<tr>
<td>CuZn-SOD</td>
<td>Middle cerebral artery</td>
<td>116</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Aorta</td>
<td>66</td>
</tr>
<tr>
<td>Parathyroid hormone-related protein</td>
<td>Aorta</td>
<td>61</td>
</tr>
<tr>
<td>Parathyroid hormone/parathyroid</td>
<td>Aorta</td>
<td>62</td>
</tr>
<tr>
<td>Protein</td>
<td>Mesenteric circulation</td>
<td>177</td>
</tr>
</tbody>
</table>

arteries in response to acetylcholine is absent. An example of responses in the carotid artery is shown in Figure 1. Relaxation of the aorta in response to A23187 (a calcium ionophore) is also absent in eNOS−/−, indicating that the findings with acetylcholine are not unique and that both receptor-mediated and receptor-independent endothelium-dependent relaxation is mediated by eNOS. Similarly, relaxation of mesenteric arteries in response to acetylcholine is greatly impaired in eNOS−/−, in which the response to acetylcholine was reduced by ∼75%. These findings provide direct evidence that endothelium-dependent relaxation of the aorta and several major arteries is mediated by eNOS (Figure 2). These results obtained in eNOS−/− mice are consistent with studies in vessels from control mice in which pharmacological inhibitors provided indirect evidence that endothelium-dependent relaxation was mediated by NO. The concept that the combination of eNOS and NO is a major mediator of responses to endothelium-dependent agonists is consistent with many studies in large arteries and resistance blood vessels from both experimental animals and humans.47-53

In addition to studies investigating the role of eNOS in responses to classic endothelium-dependent agonists, some studies have examined effects of other vasoactive stimuli in eNOS−/− mice. For example, pulmonary vasoconstriction during hypoxia is increased, and autoregulation of cerebral blood flow during reductions in arterial pressure is impaired in eNOS−/−.56

An interesting aspect of studies in eNOS-deficient mice is the phenotype observed in heterozygous eNOS-deficient mice (eNOS+/−). Although low concentrations of acetylcholine produced relaxation, higher concentrations of acetylcholine produced contraction of the carotid artery in eNOS+/− mice.31 eNOS+/− mice also have increased pulmonary vasoconstrictor responses to hypoxia. These data suggest that deletion of even one copy of the eNOS gene is sufficient to alter vascular function. The finding that eNOS+/− mice have a distinct phenotype may be relatively unique and reflect the critical importance of eNOS in the regulation of vascular tone. The findings with eNOS+/− mice also have important implications because they illustrate that only a partial loss of eNOS (as might be seen with some genetic diseases) is sufficient to alter the regulation of vascular tone.31,54

An eNOS transgenic mouse has also been described recently.57 These eNOS transgenic mice provide an example of how cellular specificity can be included in the design of a genetically altered animal as the transgene was targeted to endothelium by use of a preproendothelin-1 promoter. In these animals, basal production of NO is increased, but relaxation of the aorta in response to acetylcholine, ATPγS (another endothelium-dependent agonist), and NO donors is impaired.57 Impairment of vasorelaxation to exogenous and endothelium-derived NO after overexpression of eNOS might seem paradoxical but may reflect a downregulation of signaling mechanisms that mediate responses to NO (discussed below).

In addition to the finding that eNOS plays a major role in the regulation of vascular tone, studies in eNOS-deficient mice have highlighted other potentially important functions of eNOS in vascular biology. For example, neointimal proliferation after vascular injury is increased in the absence of eNOS and vascular smooth muscle proliferation after injury is not diminished in eNOS−/− mice.31,53

**Figure 1.** Responses of the carotid artery to acetylcholine in a wild-type mouse (eNOS+/+) (upper trace) and an eNOS−/− mouse (lower trace). Carotid rings were precontracted submaximally with U46619 before application of acetylcholine. Concentrations of acetylcholine in −log M are given above each tracing. NP indicates nitroprusside.

**Figure 2.** Schematic of effects of NO produced by eNOS on vascular tone and leukocyte adhesion. The figure is based on data obtained from genetically altered mice (eNOS- and cGKI-deficient mice). L-Arg indicates L-arginine; sGC, soluble guanylate cyclase; and cGKI, cGMP-dependent protein kinase I.
eNOS. Whether this effect is due to direct actions of NO on vascular proliferation or indirect effects, such as suppression of leukocyte migration by NO, is not known at this time. Vascular remodeling after arterial ligation is impaired, and vascular proliferation is increased in eNOS-deficient mice. Vascular remodeling during chronic hypoxia is also altered in these mice. Finally, eNOS is an important regulator of ischemia-induced angiogenesis. Thus, together, these results have provided direct evidence that endogenous NO produced by eNOS is an important regulator of vascular tone (Figure 2), growth, and remodeling as well as of the migration of vascular muscle.

**Signal Transduction**

**Receptor Function**

Several studies have examined effects of deletion of specific receptors on vascular function. This includes receptors for prostacyclin, estrogen, endothelin, angiotensin II, guanylate cyclase-A natriuretic peptide, and bradykinin (Table 1). In recent studies, the smooth muscle α-actin promoter was used to target expression of parathyroid hormone–related protein and the parathyroid hormone–related protein receptor to vascular muscle. These studies provide a second example of how cell-specific transgene expression can be included in the design of genetically altered mice (the first example was the transgenic mouse in which eNOS was targeted to endothelium by use of a preproendothelin-1 promoter, which is discussed above). In addition to providing information about the role of specific receptors under normal conditions, these studies may provide clues as to the role of these receptors under pathophysiological conditions that may have been unknown previously. For example, preliminary evidence suggests that mice deficient in expression of the bradykinin B2 receptor have increased vascular production of superoxide anion, suggesting that endogenous bradykinin, acting through this receptor, may normally suppress the production of reactive oxygen species.

**Shear Stress**

In addition to receptor-mediated responses, endothelium can produce relaxation of vascular muscle in response to some mechanical stimuli. For example, vimentin is an intermediate-filament cytoskeletal protein that is thought to be sensitive to shear stress. Importantly, flow-induced vasodilation is impaired in vimentin-deficient mice, providing direct evidence that vimentin is a key signaling protein for this mechanical stimulus. Preliminary studies in these same mice indicate that vimentin also plays a key role in vascular remodeling in response to chronic changes in blood flow.

In transgenic mice that overexpress erythropoietin, relaxation of the aorta in response to acetylcholine, but not to nitroprusside, is enhanced. The effect may be due to increased eNOS expression or activity in response to higher levels of shear stress that are present in these animals as a result of a greatly elevated hematocrit.

**Calcium Homeostasis**

Production of NO by eNOS is known to be dependent on levels of intracellular calcium. Phospholamban is a regulatory protein associated with the sarcoplasmic reticulum and is involved in intracellular calcium homeostasis. Deletion of the gene for phospholamban impairs vasorelaxation in response to acetylcholine. Because expression of phospholamban has been generally thought to be limited to cardiac, smooth, and skeletal muscle, these results were surprising and suggested an additional (previously unknown) role for the protein in endothelial. This study (Sutliff et al), which unmasked a nonpredicted result, highlights one of the advantages of studies in genetically altered mice. Similar to studies of phospholamban, targeted deletion of sarco(endo)plasmic reticulum Ca2+–ATPase, a calcium pump known to be expressed in endothelium and involved in intracellular calcium signaling, results in impairment of increases in intracellular calcium and endothelium-dependent relaxation in response to acetylcholine.

**NO-Mediated Signaling**

Vasodilation in response to NO has generally been considered to be mediated by activation of soluble guanylate cyclase and the production of cGMP. A key molecular target for cGMP is cGMP-dependent protein kinase (cGK). Two types of cGK are known to exist, cGKI and cGKII, but only cGKI is expressed at high levels in smooth muscle. Recent studies in cGKI-deficient mice have provided new evidence that vasorelaxation in response to endogenously produced NO is mediated by cGKI. For example, relaxation of the aorta in response to acetylcholine (which causes endogenous formation of NO) and to cGMP and reductions in arterial pressure in response to a donor of NO are absent in cGKI-deficient mice. Vascular muscle in cGKI-deficient mice responds normally to cAMP and adenosine, indicating that cAMP and cGMP act via different signaling pathways. The latter finding is important because some previous work has suggested that high concentrations of cAMP could potentially activate cGK. At this time, it is not known whether normal vascular responses to cAMP in cGKI-deficient mice result from expression of other compensatory mechanisms. Thus, these data provide direct evidence that cGKI is the primary mediator of relaxation of vascular muscle in response to NO and cGMP (Figure 2). This concept is consistent with earlier work, including reports suggesting that the soluble guanylate cyclase/cGMP pathway mediates relaxation of large arteries and microvessels in response to NO. In addition to regulation of vascular tone, effects of NO on vascular gene expression and cell migration, proliferation, and permeability may also be mediated by soluble guanylate cyclase, cGMP, and cGKI.

The soluble guanylate cyclase–cGKI system may be altered in the presence of other genetic manipulations or in some disease states. For example, relaxation of the carotid artery in response to nitroprusside (an NO donor) is augmented in eNOS-deficient mice (both eNOS+/− and eNOS−/− mice). Enhanced relaxation to NO in eNOS-deficient mice may represent a compensatory response to reductions in the amount of eNOS and basal NO present in blood vessels. In addition, the finding that vasorelaxation in response to NO is enhanced even in eNOS+/− mice provides additional evidence that deletion of one copy of the eNOS gene for phospholamban impairs vasorelaxation in response to acetylcholine.
gene is sufficient to alter vascular responses. Consistent with
the initial finding of enhanced responses to NO in eNOS
deficient mice is the recent finding that vasorelaxation in
response to NO was impaired in mice that overexpress eNOS
and have elevated levels of basal NO. The mechanism that
accounts for impaired responses to NO in eNOS transgenic
mice is not clear, but could potentially involve downregula-
tion of soluble guanylate cyclase or cGKI by NO.82

Studies in genetically altered mice have also provided
insight into the role of vasodilator-stimulated phosphoprotein
(VASP) in cGMP-mediated vasorelaxation. VASP is
known to be potentially phosphorylated by cGK. However,
the role of VASP in vascular function has been difficult to
study because of a lack of selective inhibitors. Recent data in
VASP-deficient mice indicate that, in contrast to cGKI,
VASP is not essential for cGMP-induced relaxation of
vascular muscle.82

Pathophysiology

Hypertension

Although many studies have now examined the effects of
various genetic alterations on regulation of arterial pressure, there
have been few studies related to vascular biology
performed in chronically hypertensive mice. Endothelium-
dependent relaxation is impaired in most studies of experi-
mental animals (nonmurine species) and of humans with
chronic hypertension.84–87 In contrast, in normal mice in
which hypertension is produced by psychosocial stress, en-
derthelium-dependent relaxation of the aorta and hindlimb has
been reported to be paradoxically increased. Although some
data are derived on endothelium-dependent responses in
genetically altered mice that are moderately hypertensive
(eNOS-deficient and cGKI-deficient mice), these data are
difficult to compare because responses to acetylcholine are
completely abolished in the absence of these essential signal-
mechanisms (as discussed above). We have begun to examine vascular function in a defined
model of hypertension, mice that overexpress human renin
(R+) and human angiotensinogen (A+) and are chronically
hypertensive.88,89 In preliminary experiments, relaxation of
the aorta and carotid arteries in response to serotonin (which produces relaxation
in this artery that is mediated by NO and activation of soluble
guanylate cyclase) is also impaired.32 The mechanism that
mediates this impairment of endothelial function may include the produc-
tion of a cyclooxygenase-derived contracting factor.91

The R+/A+ mouse represents a defined model that has
some distinct advantages over the commonly used spontane-
ously hypertensive rat (SHR) and the stroke-prone SHR. SHR
and stroke-prone SHR are models in which hypertension has
an unknown etiology and in which the genetic background is
quite dissimilar to that of the Wistar-Kyoto (WKY, the
normotensive control) rat.92 Increasing evidence suggests that
genetic background is an important additional variable in
studies of cardiovascular biology (see discussion below). The
genetic background of R+/A+ transgenic mice that were
initially studied is nearly identical to that in the control
animals because the mice in these studies were derived from
4 to 5 generations of backcross breeding to C57BL/6. With
continued backcross breeding, future studies will be able to
use mice with even greater homogeneity in the genetic
genetic background. Because previous studies examining effects of hypertension in SHR versus WKY rats have used strains that
are genetically diverse, the results are clouded by the pres-
ence of genes in the genetic background that may themselves
predispone or protect from hypertension. Thus, simple com-
parison of data obtained in SHR and WKY rats is not
optimal.92

Atherosclerosis

The most commonly used genetically altered murine model
for studies of atherosclerosis has been the apolipoprotein E
(apoE)-deficient mouse. The apoE molecule is a ligand
that mediates LDL receptor clearance of chylomicrons,
VLDLs, and other serum lipoproteins. Consequently, apoE-deficient mice fed a normal diet develop hyperlipid-
emia and atherosclerosis. The vascular lesions that
develop in these mice are quite similar to atherosclerotic
lesions in humans.93–95

Many other mouse models have been described in which
specific genetic alterations (gene overexpression or targeted
gene disruption) have altered the development or progression
of atherosclerosis.93–96 The genes that have been studied
include several involved in lipid metabolism, the immune
system, leukocyte-endothelium adhesion molecules, antioxi-
dant proteins, proteases, and thrombosis.93–96 The use of
genetically altered hyperlipidemic mice for studies of regres-
sion of atherosclerosis has also begun.97 In addition, geneti-
cally altered hyperlipidemic mice are now being commonly
used to examine effects of other interventions on the devel-
lopment of atherosclerosis.98 For example, LDL receptor–
deficient and apoE-deficient mice have been used to test the
concept that Chlamydia pneumoniae produces endothelial
dysfunction and accelerates atherosclerosis during hypercho-
lesterolemia.98–100 An even more complex approach involves
the use of multiple genetic alterations. One example is the
recent study of effects of deletion of the gene for monocyte
chemotactrant protein-1 (a chemokine) on susceptibility to
atherosclerosis in apoB transgenic mice.101 The interaction of
atherosclerosis and hypertension has also begun to be
investigated.102

In contrast to the number of studies that have examined the
effects of genetic alterations on the development of athero-
sclerosis, only a few studies have examined changes in
vascular function in these models.30,31,32 Endothelium-
dependent relaxation of the aorta is impaired in apoE-
deficient30 and combined apoE-deficient and LDL receptor–
deficient mice.27 Endothelial dysfunction is not confined to
the aorta, in view of the fact that relaxation of coronary
arteries in response to serotonin (which produces relaxation
in this artery that is mediated by NO and activation of soluble
guanylate cyclase) is also impaired.32 The mechanism that
accounts for this impairment has not been completely defined
but may involve mechanisms such as increased expression of
endothelin49 and increased production of superoxide anion.103
The basic finding that endothelial dysfunction is present in
genetically altered hyperlipidemic mice is similar to that the
finding obtained in normal mice in which hypercholesterolemia was produced by a high fat diet.13–17

The finding that endothelial dysfunction is present in atherosclerotic mice is consistent with studies of atherosclerosis in other experimental animals and in humans. In addition, genetically altered mice have begun to shed new light into the pathophysiology of atherosclerosis. Recent studies in apoE-deficient mice produced the surprising finding that a novel isoform of extracellular superoxide dismutase (extracellular SOD) is expressed in macrophages within atherosclerotic lesions.104 Studies in klotho-deficient mice have revealed that the klotho protein protects endothelial function and development of atherosclerosis.39,105 The klotho gene is involved in the suppression of several age-related phenotypes, including atherosclerosis, infertility, and osteoporosis.105 Mice that are klotho deficient may be a model of human progeroid syndromes.105

Leukocyte-Endothelial Interactions and Inflammation

Studies in genetically altered mice are also providing new insight into the molecular basis of leukocyte-endothelial interactions.106 For example, the finding that basal rolling and adhesion of leukocytes in the microcirculation is greatly elevated (≈6-fold) in eNOS-deficient mice107 provides strong evidence that NO produced by eNOS plays a major role in leukocyte-endothelial interactions under normal conditions (Figure 2).

Ischemia followed by reperfusion is known to produce endothelial dysfunction, which may contribute to tissue injury. Recent studies by Banda et al105 have shown that ischemia/reperfusion inhibits endothelium-dependent relaxation in control mice but not in animals that lack expression of the adhesion molecules CD11/CD18, intercellular adhesion molecule (ICAM)-1, or P-selectin. These studies provide strong evidence that the interaction of circulating leukocytes with endothelium after ischemia with reperfusion contributes to endothelial dysfunction.

After treatment with bacterial lipopolysaccharide (LPS), leukocyte-endothelial interactions in the cremaster microcirculation are increased in both iNOS-deficient and interleukin-10 (IL-10)–deficient mice.108,109 In addition to regulation of leukocyte-endothelial interactions, these gene products have other major vascular effects. Constrictor responses and endothelium-dependent relaxation are impaired after treatment with LPS in arteries from experimental animals and humans.110,111 In contrast, there is no impairment of vasoconstrictor responses in iNOS-deficient mice after treatment with LPS.111 and impairment of vasoconstrictor responses and endothelial function is augmented in IL-10–deficient mice.112,113 These results provided the first direct evidence that impaired vasoconstrictor responses after LPS treatment are dependent on expression of iNOS and modulated by IL-10. In addition, the findings in IL-10–deficient mice support the new concept that IL-10 protects eNOS-mediated vascular responses during inflammation. The mechanism of protection by IL-10 may involve inhibition of production of superoxide anion, which impairs endothelium-dependent relaxation.113

Cerebrovascular Injury

The use of genetically altered mice to examine the role of specific gene products in brain injury after ischemia is currently an active area of investigation. For example, focal cerebral ischemia produces more profound reductions in cerebral blood flow in the region of the ischemic penumbra and larger infarcts in eNOS-deficient mice, demonstrating the protective role of eNOS during cerebral ischemia.114

Like eNOS-deficient mice, transgenic mice that overexpress β-amyloid precursor protein have greater reductions in cerebral perfusion at the periphery of the ischemic region and enlarged infarcts after occlusion of the middle cerebral artery.114 These animals also have endothelial dysfunction in the cerebral circulation.114,115 In contrast, double transgenic mice that overexpress both β-amyloid precursor protein and CuZn-SOD have normal endothelial function, indicating that SOD can rescue the vascular defect produced by overexpression of β-amyloid precursor protein.115 Transgenic mice that overexpress CuZn-SOD are also protected against vasospasm after subarachnoid hemorrhage, suggesting that superoxide anion plays an important role in the pathogenesis of vasospasm.116

Importance of Controls

A critical issue in designing and interpreting studies using transgenic or gene-targeted mice relates to the influence of genetic background and the choice of proper control strains. In generating gene-targeted mice, the most common approach is to use embryonic stem cells from the 129 mouse strain for gene targeting and then to inject these genetically altered stem cells into blastocytes from the C57BL/6 mouse strain.5 Germ line transmission of the altered genome by breeding with a C57BL/6 mouse results in animals that are F1 of 129 and C57BL/6. Although all F1 mice are genetically identical, the heterogeneity develops when the heterozygous (+/−) F1 mice are bred to homozygosity (−/−). Although both alleles of the target loci are disrupted, the genetic background of the mice is an F2 of 129×C57BL/6. Alleles in the background of the 2 strains randomly segregate in the F2 generation. To eliminate this heterogeneity in the genetic background, a common approach is to perform backcross breeding over many generations to a common strain. After 7 generations of backcross breeding, the genetic background is >99% uniform. An alternative is to cross the original chimera with a 129 mouse to retain the inbred status of the 129 strain. Although this may seem attractive, it is rarely done because of poor reproductive performance and other abnormalities associated with the 129 strain. Because initial studies in genetically altered mice are often performed before the animals have been bred to uniformity in the genetic background, an additional common strategy is the use of littermates as controls. With littermate controls, the degree of heterogeneity in the genetic background, on the average, will be similar in control and gene-targeted animals.

It has been emphasized that controlling for genetic background is essential in studies of this type.117 It is known that gene mutations can exhibit different phenotypes on different genetic backgrounds. Thus, only if the same background is used across experiments can it be concluded that differences
in phenotype are due to the specific genetic alteration rather than differences caused by the random assortment of alleles at other loci, some of which may have nothing to do with the phenotype being examined. It has also been suggested that maintaining a mutant mouse line by homozygous inbreeding should be avoided.117

The importance of choosing proper strains of control mice becomes obvious when one examines the many examples of strain differences that have been described. For example, in relation to vascular biology, there are strain differences in resting levels of arterial blood pressure,118 in susceptibility to atherosclerosis,94,119 and ischemia,120,121 and in angiogenesis,122 vascular anatomy,123,124 and vasodilation in response to acetylcholine.125

**Limitations in Using Genetically Altered Mice**

There are limitations in the use of genetically altered mice for experimental studies. For example, a potential limitation exists in that compensation may occur in the animal either during development or later in response to deletion of the targeted gene. For example, another “redundant” gene product may replace the function of the gene that was disrupted.3 As a result, deletion of one gene product may not result in any detectable change in phenotype.

In studies of blood vessels in eNOS-deficient mice, some data are available to address this issue, and 2 lines of evidence suggest that compensation has not occurred with regard to endothelium-dependent relaxation of the aorta and other large arteries. The first line of evidence is that acute treatment with pharmacological inhibitors has an effect on responses to acetylcholine that is similar to the effect produced by deletion of the eNOS gene.31 The second line of evidence is the finding that vasorelaxation in eNOS-deficient mice in response to acetylcholine can be restored after viral-mediated gene transfer of eNOS.44 Thus, acute gene replacement (complementation) restores the phenotype to normal, suggesting that embryonic or developmental anomalies do not account for the impaired endothelium-dependent relaxation phenotype. In contrast to the aorta and other large arteries that have been studied, compensatory responses have been described in the coronary circulation and cerebral arterioles in eNOS-deficient mice.41,125 In eNOS-deficient mice, for example, the neuronal isoform of NO synthase may substitute for eNOS in mediating responses of cerebral arterioles to acetylcholine.41 These compensatory mechanisms were unmasked by means of standard pharmacological approaches, providing an example of the power of using both genetic alterations and traditional pharmacology in the same study.

The relatively small body size of mice is an additional obvious limitation for studies of blood vessels. The most commonly used method for in vitro studies of vascular function is to use vessel rings suspended in an organ bath. To date, the aorta has been by far the most frequently studied blood vessel in mice, although some studies have been performed with other mouse arteries (Tables 1 and 2). In addition, the cremaster, hindlimb, mesenteric, and cerebral circulation are used for studies of genetically altered mice in vivo. Because the aorta is not a resistance blood vessel, data obtained with use of the aorta may not always be represen-tative of mechanisms present in smaller blood vessels or in specific vascular beds. However, studies of the aorta in nonmurine species have generally been very informative over the years. There are now many examples in which findings initially obtained in aorta were later confirmed in the microcirculation. For example, the pioneering discoveries of both endothelium-derived relaxing factor and endothelin were made with use of the aorta.126,127 Moreover, the aorta is a relevant model for studies related to vascular disease that is predominantly localized in large arteries, such as atherosclerosis.

**Future Directions**

The increasing interest in the use of genetically altered mice for cardiovascular research has resulted in the application of many methods, previously used only in larger species, to pursue questions related to vascular biology in mice. These include cultivating endothelium and vascular muscle,128–130 and measurements of intracellular calcium,129,128,130 superoxide anion,103 superoxide dismutase,104 and ionic currents and membrane potentials in vascular muscle.28,131 Molecular approaches, including measurements of mRNA and protein, and research examining the effects of viral-mediated gene transfer44,132,133 and treatment with antisense oligonucleotides134 are now also being used to study murine blood vessels. In addition to the approaches and models highlighted above, genetically altered mice are being used in studies of other areas of vascular biology, including transplant atherosclerosis, hemostatic and thrombotic responses, and mechanisms of aneurysm formation.135–140

The recent identification of promoter elements sufficient to direct expression of heterologous gene products to endothelium and smooth muscle of the vasculature will clearly provide new opportunities to gain control over the spatial expression of target genes. Promoters that have been used to target expression to endothelium include preproendothelin-1, TIE2, thrombomodulin, ICAM-2, vascular endothelial cad-herin, and vascular endothelial growth factor receptor-2 (Flk-1).57,141–146 To target endothelial expression of eNOS in transgenic mice, 5’ flanking sequences from the preproendothelin-1 gene57 were used, and studies of the eNOS promoter region itself reveal that 1600-bp of the human eNOS 5’ flanking region will direct expression of the LacZ gene to the vascular endothelium.147 The smooth muscle α-actin and SM22α promoters have been used to efficiently target smooth muscle cells in transgenic mice. With these tools comes the opportunity to specifically generate models of vascular disease, to test potentially therapeutic agents, and to overexpress wild-type or dominant-negative mutants of receptors or intracel-lular signaling intermediates, antisense molecules, or reporter genes, among others.

Perhaps equally important as spatial (cell-specific) control over gene expression is temporal control. Indeed, a major potential limitation of the standard gene-targeting approach is that the phenotype observed in adult mice is the result of the lifelong loss of function of that gene product. Abnormalities caused early in development by the loss of gene function may result in a phenotype that is unrelated to its function in adults. For example, angiotensinogen-deficient mice exhibit a lethal...
its presence can now bind to the promoter of the responder (ecdysone receptor) is inactive in the absence of steroid but in the presence of a ligand-dependent transcription factor. Although the transactivator protein is nonfunctional in the absence of ligand (muristerone for the ecdysone system and doxycycline for the tet system) but becomes capable of activating transcription of a responder gene in its presence. In the ecdysone system, a coactivator is also required. The functional transactivator complex (transactivator + ligand + coactivator) can then induce the transcription of a second construct containing the transgene product of interest driven by a responsive promoter. Although the responsive promoter is not tissue specific, it can become active only in cells expressing the transactivator, which is under tissue-specific control. For this system to work, the mice must contain both constructs. B, Schematic representation of Cre-loxP system. Cre-recombinase causes an intramolecular recombination event between 2 loxP sites. In the hypothetical 4 exon gene shown, loxP sites (black arrowheads) have been engineered surrounding exon 3, which encodes a critical portion of the hypothetical protein. Exons are denoted by boxes and are numbered. In the presence of cre-recombinase, an intramolecular recombination results in the deletion of exon 3 and one of the loxP sites. This occurs when the loxP sites are oriented in the same relative direction as shown.

A number of systems are currently available to regulate the expression of genes at the temporal level. Several of these are inducible/repressible systems that are based on the use of ligand-dependent transcription factors. In short, the tetracycline (tet) or ecdysone systems use a set of both transactivator and responder constructs (Figure 3).155–157 Gene targeting is used to insert loxP sites into introns surrounding an important coding exon of the gene.158 The gene will be fully functional in the absence of cre-recombinase because the loxP sites will be eliminated after splicing. On the other hand, in the presence of cre-recombinase, a recombination event occurs at the genomic DNA level, deleting sequences between the loxP sites and rendering the gene nonfunctional. As mentioned above, spatial control over which cells will undergo the deletion is governed by the choice of cell-specific promoters driving cre-recombinase expression. Temporal control over the deletion can be achieved by using a viral vector to deliver cre-recombinase159 or by using one of the ligand-regulated promoter systems described above.157 In addition, a modification of the cre-loxP system can be used to activate the expression of a gene in a temporally controlled fashion.160

Undoubtedly, continued advances in genetic methodology that provide temporal and spatial control over gene expression, coupled with new advances in methods to study vascular function in the mouse, will provide numerous new opportunities to dissect genetic and physiological mechanisms important in vascular biology.

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**References**


113. Gunnett CA, Heistad DD, Berg DJ, Faraci FM. Superoxide anion mediates
117. Banbury Conference on Genetic Background in Mice. Mutant mice and
118. Schlager G, Weibust RS. Genetic control of blood pressure in mice.

101. Gosling J, Slaymaker S, Gu L, Tseng S, Zlot CH, Young SG, Rollins BJ,
109. Hickey MJ, Sharkey KA, Sihota EG, Reinhardt PH, MacMicking JD,

102. Sugiyama F, Haraoka S, Watanabe T, Shiota N, Taniguchi K, Ueno Y,
104. Fukai T, Galis ZS, Meng XP, Parthasarathy S, Harrison DG. Vascular

1999;30:867–872.
1224 Circulation Research
125. Godecke A, Decking UKM, Ding Z, Hirchenhain J, Bidon HJ, Godecke

124. Kitagawa K, Matsumoto M, Yang G, Mabuchi T, Yagita Y, Hori M,


134. Rosenblum WI, Murata S. Antisense evidence for two functionally active

135. Russell PS, Chase CM, Colvin RB. Accelerated atheromatous lesions in


123. Chlamydia pneumoniae infection accelerates the progression of athero-

138. Pereira L, Lee SY, Gayraud B, Andrikopoulos K, Shapiro SD, Bunton T,

139. Fay WP, Parker AC, Ansari MN, Zheng X, Ginsburg D. Vitronectin inhibits

140. Vazquez F. Nitric oxide synthase deficient mice.


86. Carmeliet P, Moons L, Colfen D. Mouse models of angiogenesis, arterial


143. Kamhi V, Zaret KS, Antel JP, Cooper DN, Anthony WF. Increased adhesion

145. Yamashita T, Matsushita K, Matsuoka K, Chiba T, Akazawa M, Ishikawa M,

148. Yang G, Kitagawa K, Matsuishi K, Mabuchi T, Kitagawa Y, Yaginaha T,

149. Thurston G, Murphy TJ, Balah P, Lindsey JR, McDonald DM. Angio-


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